Chapter 4
Bile, Bilirubin, and Cholestasis

A1. Further identify molecular causes of various forms of PFIC. Recent studies suggest that PFIC-2 may be diagnosed by immunofluorescence, whereas PFIC-3 requires gene-sequencing (Keitel V. *Hepatology*. 2005; 41:1160). The NIH-funded Cholestatic Liver Disease Consortium (CLiC) was established in 2005 and supports research to identify genetic causes of PFIC in cases not explained by defects in the *FIC1*, *BSEP* or *MDR3* genes, which are typically associated with PFIC-1, -2 and -3, respectively. (10%).

A2. Define structure-function relationships of genes involved in cholestatic liver diseases and identify potential targets for therapy. Activation of the orphan nuclear receptors CAR and PXR leads to induction of genes that protect the liver against toxic bile acids and xenobiotics; lack of these receptors worsens cholestatic liver disease in mice, making these receptors attractive targets for therapy (Wagner M. *Hepatology*. 2005; 42:420). Research on the orphan nuclear receptors in liver is an extremely active area of investigator-initiated research as well as the NIH-funded Nuclear Receptor Signaling Atlas. (10%)

A3. More fully define the normal fetal development and maturation of bile salt and bilirubin metabolic pathways. Studies in rats show that placental mRNA levels of bile acid transporters exceed those of the fetal liver until day 20 of gestation, suggesting that the fetus relies on placental clearance of bile acids (St-Pierre MV. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R1505). (10%)

B1. Define whether polymorphisms of major bile transporters are involved in drug-induced cholestatic liver disease. The NIH-sponsored DILIN network is assembling a cohort of patients and controls with drug-induced liver disease for genetic studies of susceptibility to this injury. DNA samples from patients will be tested for polymorphisms of drug-metabolizing enzymes and bile regulatory transporters and receptors. (0%)

B2a. More fully elucidate the normal pathways of bile salt, lipid, and organic solute uptake, synthesis, transport, and secretion in hepatocytes. The discovery of Ostα-Ostβ to be the bile acid transporter through the basolateral membrane in the mouse ileum and its identification in human ileum, kidney and liver has helped to complete our understanding of the enterohepatic circulation of bile salts (Dawson PA. *J Biol Chem*. 2005;280: 6960; Ballatori N. *Hepatology* 2005;42:1270). (20%)

B2b. Define the pathways and regulation of hepatic cholesterol synthesis and secretion. This is an area of active investigator-initiated research. In the past year, the scavenger receptor class B type I (SR-BI) has been shown to be a regulator of cholesterol efflux from macrophages for excretion by the liver into bile and feces (Zhang YZ. *J Clin Invest* 2005;115:2870) Furthermore, the ABCG1 and ABCA1 transporters act synergistically in mediating cholesterol efflux from macrophages and incorporation into HDL particles (Gelissen IC. *Arterioscler Thromb Vasc Biol* 2005; In press). Biliary cholesterol secretion is regulated

largely through the ABCG5/G8 transporters, which in turn are regulated by the nuclear receptors LXR, FXR and PXR (Yu L. *J Biol Chem* 2005;280:8742). (10%)

B3. Develop drug therapy that stimulates bilirubin metabolic pathways or interferes with bilirubin production in the newborn. Advances in this area await further elucidation of the role of orphan nuclear receptors and the pathways of bilirubin and bile acid metabolism in the newborn and development of agents that target these pathways. (0%)

C1. Define molecular basis of pruritus and identify targets for potential therapies. Clinical and molecular studies indicate that rifampin enhances bile acid detoxification and bilirubin export, while ursodeoxycholic acid (UDCA) stimulates bile acid export, suggesting that this combination might be effective in treating cholestasis and pruritus (Marschall HU *Gastroenterology* 2005; 129: 476-85). Small grants of innovative therapies for pruritus are encouraged in the ongoing program announcement on "Small Clinical Grants in Digestive Diseases, Nutrition and Obesity" (PA-04-088). (10%)

C2. Define the molecular basis and means of screening for or diagnosing acquired or adult forms of cholestatic liver disease such as cholestasis of pregnancy, sepsis, or total parenteral nutrition. Two recent studies from Europe have shown that UDCA improves the biochemical laboratory tests and pruritus in women with intrahepatic cholestasis of pregnancy (Glantz A *Hepatology*. 2005;42:1399; Kondrackiene J. *Gastroenterology* 2005;129:894). (10%)

C3. Develop effective gene therapy for at least one form of severe, neonatal cholestasis or hyperbilirubinemia. Diseases of particular focus include Crigler-Najjar syndrome and Byler disease (PFIC-1); without liver transplantation, both of these diseases are usually fatal during childhood. Progress in gene therapy is encouraged by the NIH in several requests for applications and program announcements and through grants that use gene therapy centers. (0%)

Figure 6. Estimated Progress on Bile, Bilirubin, and Cholestasis Research Goals, 2005 (Year 1)